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EXAMINER	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/590,897	CARMI ET AL.	
	Examiner	Art Unit	
	MARIA LEAVITT	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *13 January 2011*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *72-102* is/are pending in the application.

4a) Of the above claim(s) *72-85 and 94-101* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *86-93 and 102* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *28 August 2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *01/13/11;08/28/06*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

This action is in response to papers filed January 13, 2011. Applicants' response to the restriction requirement of October 10, 2010 has been entered. Currently, claims 72-102 are pending. Claim 102 has been added and claims 72-85, 87-90 and 94-101 haven been withdrawn by Applicants' amendment filed on 01-13-2011.

Election/Restrictions

Applicants' election with traverse of Group VII, i.e. 86, 91-93 and new claim 102, directed to a method for treating a disease comprising: a) providing a composition comprising a DNA molecule comprising a nucleotide sequence encoding at least one enzyme, the at least one enzyme is capable of mediating site-specific excision of a gene fragment flanked between two recombination sites at a defined genomic locus, wherein at least one recombination site is an asymmetric recombination site; b) transforming a cell with the composition; and c) proliferating the transformed cells ex vivo, wherein the cell is autologous, in Applicants' response filed on 01/13/2011 is acknowledged. Additionally, election of microparticles as the species of a carrier for the composition is acknowledged.

At page 11 of the remarks filed on 01-13-2011, Applicants essentially argue that claims 87 to 90 should be examined together with group VII, particularly because the specification discloses at paragraph [0130] of the published application that the claimed composition may be used to treat a variety of diseases including excision of HIV provirus from the genome of infected cells. Additionally, Applicants point out that claim 90 has not been included into a restricted group. The above arguments have been fully considered and deemed persuasive. Accordingly, the examiner has withdrawn the restriction requirement between Group VII, and

claims 87-90. Claims 72-85 and 94-101 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim.

The election requirement is deemed proper and is therefore made **FINAL**.

Please note that after a final requirement for restriction, the Applicants, in addition to making any response due on the remainder of the action, may petition the Commissioner to review the requirement. Petition may be deferred until after final action on or allowance of claims to the invention elected, but must be filed not later than appeal. A petition will not be considered if reconsideration of the requirement was not requested. (See § 1.181.). The instant claims have been examined commensurate in scope of the elected invention and the species of the invention.

Therefore, claims 86-93 and 102 are under current examination to which the following grounds of rejection are applicable.

Claim Rejections - 35 USC § 112- Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

Claims 86-93 and 102 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 86 is rendered vague and indefinite by the recitation of the phrase "the isolated DNA molecule". There is insufficient antecedent basis for this limitation in the claim. As such, the metes and bounds of the claim cannot be determined.

Claim 86 is indefinite in their recitation of the phrase "at least one recombination site is an asymmetric recombination site" since it is unclear in reference to what recombination site the asymmetric recombination site is defined and the extent the asymmetry. Is the recombinant site defined in relation to other symmetric recombination flanking site? Is the asymmetric recombination site related to the same flanking symmetric site in another DNA molecule? Is the asymmetric sequence related to two symmetric flanking sequences of a gene fragment? As such, the metes and bounds of the claims cannot be determined.

Additionally, claim 86 is incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. While all of the technical details of a method need not to be recited, the claims should include enough information to clearly and accurately describe the invention and how it is to be practice. The only disclosed steps in claim 86 are: a) providing a composition comprising a DNA molecule able to mediate site-specific recombination and providing said composition to a subject thereby obtaining a site specific excision of a gene fragment from a determined genomic locus. It is not apparent as to under what structural or functional parameters the excision of a gene fragment from a determined genomic locus is indicative or correlative to the preamble of the claims.

Claims 87-93 and 102 are rejected insofar as they depend on claim 86.

Claim Rejections - 35 USC § 112 - written description

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 86-93 and 102 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claims contain subject matter which was not described in the specification in such a way as to reasonably convey to any person skilled in the art to which it pertains, or with which it is most nearly connected, at the time the application was filed, that the inventor, at the time the application was filed, had possession of the claimed invention.

Claims 86-93 and 102 as best understood, are readable on a genus of nucleotide molecules encoding a recombinase enzyme able to mediate site-specific excision of a gene fragment flanked between two recombination sites, wherein at least one recombination site is an asymmetric recombination site, wherein the undisclosed enzyme is able to recognize any canonical unique asymmetric site in a DNA sequence and be able to functional excision of a gene fragment flanked between two recombination sites, wherein the genus of nucleic acids is not claimed in a specific biochemical or molecular structure that could be envisioned by one skilled in the art at the time the invention was made. The specification and claims do not place any limits on the location and number of modifications in the DNA molecule encoding the undisclosed recombinase so as to generate a functional enzyme for site specific recombination and excision of a gene fragment flanked between two recombination sites. The specification and claims do not place any limits on the location amino acid changes in the encoded undisclosed enzyme or whether the amino acid changes are clustered or separated.

To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail such that the Artisan can reasonably conclude that the inventor(s) had possession of the claimed invention. Such possession may be demonstrated by

describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and/or formulae that fully set forth the claimed invention. Possession may be shown by an actual reduction to practice, showing that the invention was "ready for patenting", or by describing distinguishing identifying characteristics sufficient to show that Applicant was in possession of the claimed invention (January 5, 2001 Fed. Reg., Vol. 66, No. 4, pp. 1099-11). Moreover, MPEP 2163 states:

> [A] biomolecule sequence described only by a functional characteristic, without any known or disclosed correlation between that function and the structure of the sequence, normally is not a sufficient identifying characteristic for written description purposes, even when accompanied by a method of obtaining the claimed sequence.

Applicants merely provides on example of a Cre recombinase wherein two Cre variants that recognize lox M7 (e.g.., lox M7 is a loxP variant), termed: 1) CM1 (corresponding to C2(+)#1), and 2) CM2 (corresponding to C2(+/−)#4), were selected from libraries of Cre variants generated by targeted random mutagenesis (page 31, lines 14-17). Moreover, the specification discloses specific asymmetric lox variants sites identified as loxP-M7 and M7-loxP, wherein each asymmetric site was composed of a wild-type loxP half-site and lox M7, and each member of the pair of lox sites was positioned in direct orientation relative to the other, flanking an intervening ~1-kb DNA fragment (page 32, lines 21-33; Fig. 1A). Recombination of asymmetric lox sites in vitro was demonstrated for wt Cre, CM1 (a Cre-related recombinase efficient on loxP and lox M7 substrates because relaxed substrate specificity in vitro), CM2 (e.g. a Cre-related recombinase possessing selective binding specificity for its respective cognate lox M7, e.g., 40-fold higher specificity for lox M7 than loxP) and a wt Cre-Cm2 mixture. The Cre variants CM1, CM2 and the wt Cre-Cm2 mixture were used at different enzyme concentration

with a constant concentration of loxP-M7 DNA substrate in binding assays (page 33, lines 1-15; Fig 3A-3C). The specification concludes that in vitro recombinant activity supports two conclusions: (1) A single Cre-related recombinase with relaxed substrate specificity that functions equally efficiently on loxP and lox M7 substrates (i.e. CM1) can efficiently catalyze recombination of the chimeric asymmetric substrate, (2) a combination of two different Cre variants (wt and CM2) possessing selective binding specificities for their respective cognate lox half-sites (loxP or lox M7 respectively) can efficiently catalyze recombination of the chimeric loxP-M7 asymmetric substrate i.e., loxP-M7 and M7-loxP asymmetric substrates (page 34, lines 4-10). Though the specification disclose at pages 36-37, in Table 3, potential lox-variant sites within mammalian genomes generated by computational analysis, the specification as filed is silent about regions or domains of the genus of undisclosed recombinant enzymes encoded by a DNA molecule essential for mediating site-specific excision of a gene fragment flanked between two recombination sites, identity of the base pairs in the palindromic or asymmetrical sequences of each flanking site recognized by the recombinase or sites of the recombinase that are critical for selective binding specificities to their cognate recombinant DNA site in a predetermined genomic locus.

At the time the invention was made, it was well known in the art that certain positions in the sequences of peptides/proteins are critical to the protein's structure/function relationship, particularly, various sites or regions directly involved in binding, recombination activity and in providing the correct three-dimensional spatial orientation of binding and active sites. Particular regions may possess critical determinants for binding specifity to their respective cognate half sites. These regions can tolerate only relatively conservative substitutions or no substitutions

(Guo et al.,.Proc Natl Acad Sci 2004 101:9205-10; p. 9209, col. 1, last paragraph). The skilled artisan understands that one nucleotide change in a DNA molecule or one amino acid change in the polypeptide encoded by the DNA molecule could result in the loss of its biological recombination activity as demonstrated in the generation of sickle-cell anemia wherein on specific amino acid mutation gave rise to the inherited disease (Voet et al., Biochemistry John Wiley and Sons, 1990, p. 126-129). Even single-nucleotide polymorphism without affecting the amino acid sequence can affect folding of the protein and thus alter its function (Kimchi-Sarfaty et al., 2007, Science, pp. 525-528; p. 527, col. 3, last paragraph). Further, Rudinger (in Peptide Hormones, Parsons (ed.), University Park Press: Baltimore, MD, pp. 1-7, 1976) discloses that even for peptide hormones (e.g. 9-amino acids in length), which are smaller than the instant Cre protein, one cannot predict variant amino acid sequences for a biologically active polypeptide. Rather one must engage in "case to case painstaking experimental study" to determine active variants (see page 7). Though the Wt Cre has been shown to tolerate changes within the 8-bp spacer region of the 34-bp cognate site (termed LoxP) as illustrate in Table 2 of the specification as filed indicating that Cre target specifity can be altered to recognize moderately altered DNA target sites, this may not be sufficient, as the ordinary artisan would immediately recognize that an active or binding site of recombinase must assume the proper three-dimensional configuration to be active, which conformation is dependent not only upon residues that recognize the asymmetrical 8-bp spacer region of LoxP, but also on residues in the Cre recombinase which binds and recombine more divergent target sites. No structural characteristics for any potential enzyme obtained from the computational screening method have been provided beyond the characteristics of substrates having domains similar to lox-like substrates and Cre variants (wt

and CM2) possessing selective binding specificities for their respective cognate lox half-sites (loxP or lox M7 respectively) to catalyze recombination of the chimeric loxP-M7 asymmetric substrates. There is not structure/function relationship taught at all in the specification for the undisclosed recombinant enzymes encoded by a DNA molecule essential for mediating site-specific excision of a gene fragment flanked between two recombination sites. The mere description of Cre- variants: CM1, CM2 and a wt Cre-Cm2 mixture efficiently able to recombine the four combinations and positions of loxP-M7 and M7-loxP DNA chimeric substrates is not deemed to be descriptive of the complete structure of a representative number of species encompassed by the claims, as one of skill in the art cannot envision all the nucleotide sequences encoding at least any recombinant enzyme able to mediate site-specific excision of a gene fragment flanked between two recombinant sites wherein one recombinant site is an asymmetric recombination site from a predetermined genomic locus.

In conclusion, although sufficient description is given for a composition comprising the wt Cre-CM2 mixture and CM1 which efficiently catalyze recombination of the corresponding loxP-M7, M7-loxP asymmetric substrates, this limited information is not deemed sufficient to reasonably convey to one skilled in the art that Applicant is in possession of a genus of nucleic acid sequences encoding recombinant enzymes for mediating site-specific excision of a gene fragment flanked between two recombination sites. Thus it is concluded that the written description requirement is not satisfied for the claimed genus.

Claim Rejections - 35 USC § 112- First paragraph- enablement

Claim 86-93 and 102 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not

described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The specification does not reasonably provide enablement for claims directed to a method for treating a disease comprising: a) providing a composition comprising a DNA molecule encoding a least one enzyme able to mediate site-specific excision of a gene fragment flanked between two recombination sites, and b) administering the composition to a subject thereby obtaining site-specific excision of the gene fragment from a predetermined genomic locus, wherein the composition further comprises a carrier operably linked to the DNA molecule.

The specification does not enable any person skilled in the art to which it pertains or with which it is most nearly connected, to use the invention commensurate in scope with this claim. Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC 112, first paragraph, have been described by the court in In re Wands, 8 USPQ2d 1400 (CA FC 1988). Wands states at page 1404,

> "Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in Ex parte Forman. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

The claims when given the broadest reasonable interpretation encompass a method for treating any disease in a subject including a human subject comprising administration of a composition and a carrier comprising a DNA encoding any recombinant enzyme able to recognize a gene fragment flanked between two recombinant sites so as to excise said gene fragment, said composition able to reach a genomic locus by any route of administration

whereby said administration achieves a therapeutic effect. Thus, the claimed invention is directed to an in vivo therapy method for treating or protecting a patient exhibiting an unidentified number of disorders. Additionally, the claims broadly embrace a genus of nucleotide molecules encoding any recombinase enzyme able to mediate site-specific excision of a gene fragment flanked between two recombination sites, wherein at least one recombination site is an asymmetric recombination site, wherein the enzyme is able to recognize any canonical unique asymmetric site in a DNA sequence, wherein the genus of nucleic acids is not claimed in a specific biochemical or molecular structure that could be envisioned by one skilled in the art at the time the invention was made. The specification provides insufficient data to enable claims directed to the method as broadly claimed. Specific considerations such as the correlation between the specific excision of a gene fragment from a predetermined genomic locus, and administration to a subject in need thereof being so as to achieve a therapeutic effect and the predictability of a genus of nucleic acid encoding for at least one recombinant enzyme being able to target and excise a gene fragment from its chromosomal integration site have to be addressed for an effective in vivo therapy treatment. The detail of the disclosure provided by the Applicant, in view of the prior Art, must encompass a wide area of knowledge to enable one of ordinary skill in the art at the time of the invention to practice the invention without undue experimentation. However, as it will be discussed below this undue experimentation has not been overcame by the as-filed application.

Applicants merely provides on example of a Cre recombinase wherein two Cre variants that recognize lox M7 (e.g.., a loxP variant), termed: 1) CM1 (corresponding to C2 (+)#1), and 2) CM2 (corresponding to C2(+/−)#4), were selected from libraries of Cre variants generated by

targeted random mutagenesis (page 31, lines 14-17). Moreover, the specification discloses specific asymmetric lox variants sites identified as loxP-M7 and M7-loxP, wherein each asymmetric site was composed of a wild-type loxP half-site and lox M7 and each member of the pair of lox sites was positioned in direct orientation relative to the other, flanking an intervening ~1-kb DNA fragment (page 32, lines 21-33; Fig. 1A). Recombination of asymmetric lox sites in vitro was demonstrated for wt Cre, CM1 (a Cre-related recombinase efficient on loxP and lox M7 substrates because relaxed substrate specificity in vitro), CM2 (e.g. a Cre-related recombinase possessing selective binding specificity for its respective cognate lox M7, e.g., 40-fold higher specificity for lox M7 than loxP) and a wt Cre-Cm2 mixture at different concentrations with a constant concentration of the loxP-M7 DNA substrate (page 33, lines 1-15). The specification concludes that in vitro recombinant recombination activity support two conclusions: (1) A single Cre-related recombinase with relaxed substrate specificity that functions equally efficiently on loxp and lox M7 substrates (CM1) can efficiently catalyze recombination of the chimeric asymmetric substrate, (2) a combination of two different Cre variants (wt and CM2) possessing selective binding specificities for their respective cognate lox half-sites (loxP or lox M7 respectively) can efficiently catalyze recombination of the chimeric loxP-M7 asymmetric substrate (i.e., loxP-M7, M7-loxP asymmetric substrates (page 34, lines 4-10). Though the specification disclose in Table 3, potential lox-variant sites within mammalian genomes generated by computational analysis, the specification is silent about regions or domains of the genus of undisclosed recombinant enzymes encoded by a DNA molecule essential for mediating site-specific excision of a gene fragment flanked between two recombination sites, identity of the base pairs in the palindromic or asymmetrical sequences of

each flanking site recognized by the recombinase or sites of the recombinase that are critical for selective binding specificities to their cognate recombinant DNA site in a predetermined genomic locus. In relation to clinical applications of recombinant enzymes to target and excise a gene fragment from a predetermined genomic locus, the application discloses potential natural asymmetric lox sites in several mammalian genomes based on the 33 tolerable spacers of the LoxP (e.g., locus of X-over P1), with several hundred asymmetric lox-like sites in each genome (Table 3 of the specification as filed), specifically, FIG. 5 and page 39 illustrates lox-LTR sequences and replacement of the LoxP sites with LTR sequences. Though the specification contemplates selection of Cre mutants that facilitate genetic antiviral therapy against HIV by excising the viral genome flanked between viral LTR recombination sites, the specification is silent about how to therapeutically target any human disorder so as to efficiently have a therapeutic effect.

Regarding the claimed invention drawn to the use of any type of human disorder, applicant' claims as written encompass a genus of variants of therapies including somatic or/and acquired disorders, e.g., hypercholesterolemia, emphysema, atherosclerosis, cancer, sickle cell disease, severe lymphocyte disorder, immunodeficiency disorders, and as such the in vivo gene-transfer-mediated treatment and/or vaccination have to determine whether the disorder is inherited or acquired in order to select form a diversity of therapy approaches. In the case of inherited disorders, the insertion of a new gene that ultimately corrects a deficiency requires that the new gene product is present in sufficient amount to achieve a therapy. By contrast, in acquired diseases, since a particular gene or unrelated biochemist process may contribute to the disorder, the approach to therapeutically target a human disorder is complex by the number of

factors to be considered and often the incomplete understanding of the pathology of the acquired disease. Thus, the instant claims are broadly drawn to a genus of differing diseases in terms of their pathologic mechanisms and therapeutic end points. Accordingly, ach therapeutic approach should encompass the specifics for the human disorder being contemplated. Hence the application of gene transfer technology is complex and the ability to develop clinically efficacious therapies is limited by problems that still plague all gene therapy strategies (see Goodman and Gilman's The Pharmacological Basis of Therapeutics, 1996, p.81; The Scientist, 2006; p. 36, whole document ; Patil et al ; The AAPS Journal 2005. pp. 1407-1057). In relation to administration of a composition to a subject comprising a DNA molecule by any route, the art at the time of filing teaches that systemic barriers (e.g., degradation of DNA in plasma, inability of DNA to target specific organs, largely ineffective administration via the oral route) and cellular DNA barriers (e.g. endosomal escape of DNA, lysosomal degradation, cytoplasmic stability of DNA, translocation of DNA to the nucleus) affect expression of non-viral gene expression constructs. For example, the inability to achieve effective gene transfer in differentiated; non-dividing cells possessing an intact nuclear membrane may pose the most important limitation for successful nonviral gene transfer (Zabner et al., JBC, 270, 18997-19007, 1995, p. 19005, col. 2, paragraphs 1 and 2). Moreover, Lechardeur et al. (Gene Ther. 6:482-497, 1999) describes metabolic instability of plasmid DNA in the cytosol as a further barrier to gene transfer. The specification as filed fails to provide particular guidance to resolve the known unpredictability in the art associated with treatment of any disease in view of the complexity of treating any disorder with gene therapy. Accordingly, the quantity of experimentation required to practice the methods as claimed would require the de novo determination of effective target sites,

modes of delivery, safe administration of the composition comprising a DNA molecule to target appropriate cells and/or tissues in a patient having any somatic or/and acquired disorders, and further whereby treatment effects are provided for the claimed condition. Note that at the time the invention was made, the manipulation of the loxp/Cre system under a number of different configurations and schemes, wherein Cre expression and/or transgene expression may be controlled by an interplay of inducible and tissue-specific promoters specifically selected to control the time and place of the transgene expression in specific cells, tissues, and/or at specific developmental stages was known in the art. Numerous examples, including Akimoto et al.,(2004) Li et al., (2005) Stricklett et al.(1999) , Lakso et al., (1992) Gossen et al., (2002) specifically enable the use of such constructs and clearly exemplify the versatility of the system. However, the immediate therapeutic approach of using an engineered Cre recombinase and other variants in any clinical setting is still unpredictable (see, Sarkar et al., 2007, Science pp. 1912-1915; p. 1915, col. 3). Thus it is clear that the therapeutic treatment of widely divergent diseases is highly complex, and requires consideration of numerous factors. In contrast the instant specification has only provide limited in vitro characterization of composition comprising the wt Cre-CM2 mixture and CM1 efficiently catalyzing recombination of the corresponding chimeric loxP-M7, M7-loxP asymmetric substrates, including asymmetric lox-like lox-LTR. Hence, it would be undue experimentation for one of ordinary skill in the Art to determine, for any particular subject in need of treatment, to practice the invention as presently claimed.

Regarding the claimed invention drawn to a genus of nucleotide molecules encoding a recombinase enzyme able to mediate site-specific excision of a gene fragment flanked between two recombination sites, wherein at least one recombination site is an asymmetric recombination

site, wherein the enzyme is able to recognize any canonical unique asymmetric site in a DNA sequence, the specification does not teach critical regions or domains of the nucleic acids encoding any enzyme able to recognize and bind a DNA recombination site so as to mediate site-specific excision. Though the specification discloses in Table 3 potential lox-variant sites within mammalian genomes generated by computational analysis, the specification is silent about regions or domains of the genus of recombinant enzymes encoded by a DNA molecule essential for mediating site-specific excision of a gene fragment flanked between two recombination sites, identity of the base pairs in the palindromic or asymmetrical sequences of each flanking site recognized by the recombinase or sites of the recombinase that are critical for selective binding specificities of their cognate recombinant DNA site in a predetermined genomic locus in a human subject. At the time the invention was made, it was well known in the art that certain positions in the sequences of peptides/proteins are critical to the protein's structure/function relationship, particularly, various sites or regions directly involved in binding, recombination activity and in providing the correct three-dimensional spatial orientation of binding and active sites. Particular regions may also be critical determinants of antigenicity. These regions can tolerate only relatively conservative substitutions or no substitutions (Guo et al.,. Proc Natl Acad Sci U S A. 2004 101:9205-10; p. 9209, col. 1, last paragraph). The skilled artisan understands that one nucleotide change in a DNA molecule or one amino acid change in the polypeptide encoded by the DNA molecule could result in the loss of its biological activity as demonstrated in the generation of sickle-cell anemia wherein on specific amino acid mutation gave rise to the inherited disease (Biochemistry John Wiley and Sons, 1990, p. 126-129). Even single-nucleotide polymorphism without affecting the amino acid sequence can affect folding of the protein and

thus alter its function (Kimchi-Sarfaty et al., 2007, Science, pp. 525-528; p. 527, col. 3, last paragraph). Further, Rudinger (in Peptide Hormones, Parsons (ed.), University Park Press: Baltimore, MD, pp. 1-7, 1976) discloses that even for peptide hormones (e.g. 9-amino acids in length), which are smaller than the instant Cre protein, one cannot predict variant amino acid sequences for a biologically active polypeptide. Rather one must engage in "case to case painstaking experimental study" to determine active variants (see page 7). Though the Wt Cre has been shown to tolerate changes within the 8-bp spacer region of the 34-bp cognate site (termed LoxP) as illustrate in Table 2 of the specification as filed indicating that Cre target specifity can be altered to recognize moderately altered DNA target sites, this may not be sufficient, as the ordinary artisan would immediately recognize that an active or binding site of recombinase must assume the proper three-dimensional configuration to be active, which conformation is dependent not only upon residues that recognize the asymmetrical 8-bp spacer region of LoxP, but also on residues in the Cre recombinase which binds and recombine more divergent target sites. No structural characteristics for any potential enzyme obtained from the computational screening method have been provided beyond the characteristics of substrates having domains similar to lox-like substrates and Cre variants (wt and CM2) possessing selective binding specificities for their respective cognate lox half-sites (loxP or lox M7 respectively) to catalyze recombination of the chimeric loxP-M7 asymmetric substrates. There is not structure/function relationship taught at all in the specification for recombinant enzymes encoded by a DNA molecule essential for mediating site-specific excision of a gene fragment flanked between two recombination sites. The mere description of Cre- variants: CM1, CM2 and a wt Cre-Cm2 mixture efficiently able to recombine the four combinations and positions of symmetric

loxP-M7 and M7-loxP DNA chimeric substrates is not deemed to be descriptive of the complete structure of a representative number of species encompassed by the claims, as one of skill in the art cannot envision all the nucleotide sequences encoding at least a recombinant enzyme able to mediate site-specific excision of a gene fragment flanked between two recombinant sites wherein one recombinant site is an asymmetric recombination site from a predetermined genomic locus.

As the result, given the unpredictability of the art and the lack of working example in the instant specification, particularly because the specification does not described the breadth of the DNA molecule variants claimed, it would have required undue experimentation to determine alternative sequences meeting the claims requirements that could have the claimed recombination activity.

As set forth in In re Fisher, 166 USPQ 18 (CCPA 1970), compliance with 35 USC 112, first paragraph requires:

> that scope of claims must bear a reasonable correlation to scope of enablement provided by specification to persons of ordinary skill in the art; in cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws; in cases involving unpredictable factors, such as most chemical reactions and physiological activity, scope of enablement varies inversely with degree of unpredictability of factors involved.

In Amgen Inc. v. Chugai Pharmaceutical Co. Ltd., 18 USPQ2d 1016 (Fed. Cir. 1991), the court ruled that a claim to a large genus of possible genetic sequences encoding a protein with a particular function that needs to be determined subsequent to the construction of the genetic sequences may not find sufficient support under 35 USC 112, 1st para., if only a few of the sequences that meet the functional limitations of the claim are disclosed and if undue

experimentation would be required of one skilled in the art for determining other genetic sequences embraced by the claim. This is the case here, where specification discloses one putative Cre-variant: CM1 and a wt Cre-Cm2 mixture efficiently able to recombine the four combinations and positions of asymmetric loxP-M7 and M7-loxP DNA chimeric substrates; however the specification is silent about any other variants of able to exhibit the recombination activity of CM1 and a wt Cre-Cm2 mixture.

The instant issue is whether or not the prior art and the as-filed application provides sufficient guidance and the degrees of predictability as to the structural and functional correlation between the energy emitted by any therapeutic agent and the delivery of said energy to achieve a therapeutic effect. A close review of the entire specification and the prior art does not appear to provide such guidance, particularly in view of the nature and complexity of treating a genus of undetermined somatic or/and acquired disorder with gene therapy, much less by administration said composition comprising a DNA molecule by any route, said DNA molecule encoding any recombinase able to specifically excise a gene fragment flanked between two recognition sites from a predetermined genomic locus, so as to achieve a therapeutic effect. Without such guidance in the specification and the lack of correlative working examples, the claims would require an undue amount of experimentation without a predictable degree of success on the part of the skilled artisan.

Conclusion

Claims 87-93 and 102 are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria Leavitt whose telephone number is 571-272-1085. The examiner can normally be reached on M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach, Ph.D can be reached on (571) 272-0739. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Maria Leavitt/

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Primary Examiner, Art Unit 1633